Comparison of Four Enzyme Immunoassays With a Western Blot Assay for the Determination of Type-Specific Antibodies to Herpes Simplex Virus

Thomas B. Martins, I(ASCP),[1] Ryan D. Woolstenhulme, MT(ASCP),[1] Troy D. Jaskowski,[1] Harry R. Hill, MD,[1,2] and Christine M. Litwin, MD[1,2]

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Abstract

Most current enzyme immunoassays (EIAs) differentiate inadequately between types 1 and 2 herpes simplex virus (HSV) antibodies since significant cross-reactivity exists. We compared 4 IgG type-specific EIAs using a Western blot assay for resolution of discrepant results. The Diamedix had sensitivities of 100% for types 1 and 2 but specificities of only 71% and 61%, respectively. The cross-reactivity rate was 82% in positive samples tested. For HSV types 1 and 2, the Zeus sensitivities were 92% and 98%, respectively; specificities were 72% and 79%, respectively; the cross-reactivity rate was 54%. For HSV types 1 and 2, the Wampole sensitivities were 98% and 95%, respectively; specificities were 68% and 85%, respectively; the cross-reactivity rate was 47%. For HSV types 1 and 2, the Meridian sensitivities were 98% and 90%, respectively; specificities were 96% and 100%, respectively; no cross-reactivity was found between positive samples tested. While the Diamedix, Zeus, and Wampole assays showed good sensitivity, they lacked type specificity. The Meridian EIA offers the highest specificity along with no observed cross-reactivity. This EIA may be an easier, reliable alternative to Western blot for the determination of HSV type-specific antibodies.

Most herpes simplex infections are not life-threatening, except for encephalitis and neonatal infections. Neonatal herpes can be a serious disease, often involving the central nervous system, with a mortality rate of 70% in untreated persons.[1] Herpes simplex encephalitis is one of the most devastating of all herpes simplex virus (HSV) infections and is considered the most common cause of sporadic, fatal encephalitis in the United States.[2]

Many HSV type 2 infections are subclinical and may be undiagnosed. Most sexual transmission[3,4] and maternal transfer to infants[5] occurs because people are unaware that they have genital herpes. An important factor in the prevention of neonatal herpes, which is most often due to type 2 infection, is the identification of pregnant women with asymptomatic infections. Perhaps even more important may be the identification of type 1 or type 2 seronegative pregnant women who are near term and who have seropositive partners. Infants born to seronegative mothers acquiring a primary case of genital HSV type 2 infection are at a greater risk of acquiring neonatal herpes than infants born to seropositive mothers with recurrent genital herpes.[5] Primary cases late in gestation are particularly dangerous for a number of reasons: infants potentially may be exposed to a larger viral inoculum; the cervix is much more frequently involved, posing a greater risk since transmission usually occurs at the time of delivery; and the infant lacks transplacentally acquired antibodies.[5] In such cases, antiviral intervention and counseling may help prevent neonatal herpes.[6,7]

Definitive diagnosis of genital herpes is fundamental to the management of patients and the development of strategies to prevent transmission to partners and neonates.[8] Detection of herpesvirus-specific antibodies permits diagnosis of an infection when virologic methods such as culture, antigen

detection, and polymerase chain reaction are impractical, too costly, or yield negative results.[9] Serology is an effective way to diagnose subclinical HSV type 2 infections, but most available HSV serologic tests are of limited value because they cannot accurately discriminate between type 1 and type 2 HSV antibodies. Because herpesvirus types 1 and 2 share many common antigens, there is considerable cross-reactivity among most type 1 and type 2 enzyme immunoassays (EIAs) based on whole viral proteins.[10] Therefore, determining whether a positive result in both HSV type 1 and 2 assays represents cross-reactive antibodies associated with a single infection or true dual infection is difficult. Truly type-specific serologic assays would permit accurate identification of asymptomatic HSV type 2 infection in patients with or without preexisting antibodies to type 1 HSV. Such tests could provide useful information in the diagnosis of subclinical or undiagnosed HSV type 2 infections, as well as aid in the prevention of maternal transfer of HSV to the neonate.

Several type-specific antibody tests are available that are based on Western blot (WB) analysis and purified type-specific glycoprotein G from types 1 and 2 HSV. WB analysis has the ability to distinguish between types 1 and 2 antibody and is considered the traditional "gold standard" for differentiating between type 1 and type 2 antibodies. Owing to its cumbersome nature and the lack of a readily available antigen source, WB analysis is not a practical option for most clinical laboratories. Recently, type-specific purified glycoproteins have been used to develop reliable immunoassays to detect antibodies to HSV. The HSV type 1 glycoprotein G (gG-1) and HSV type 2 glycoprotein (gG-2) show little sequence homology. The common cross-reactive sequences are found primarily in the leader sequence, which is lost during processing of the proteins after translation, and in the membrane anchor region, which has limited immunogenicity owing to its sequestration in the infected cell membrane.[8,11,12] Several commercial assays based on these proteins are available.

Materials and Methods

Samples

In these studies, serum samples from 158 patients submitted to our reference laboratory for HSV IgG EIA testing were examined. Specimens were stored at –20°C before testing and at 2°C to 8°C between individual assays. All samples were free of substantial lipemia, hemolysis, and bacterial contamination.

Enzyme Immunoassays

We evaluated 4 commercially available type-specific EIAs using WB analysis, the accepted gold standard, for resolution of discrepant results. Three of the assays (from Diamedix, Miami, FL; Wampole Laboratories, Cranbury, NJ; and Zeus Scientific, Raritan, NJ) are based on inactivated viral particles, whereas the assay from Meridian Diagnostics (formerly Gull Laboratories), Cincinnati, OH, is based on affinity-purified glycoprotein types 1 and 2 HSV antigens (F strain). All EIAs were labeled "For in vitro diagnostic use" according to US Food and Drug Administration regulations. Any sample results that were not in total agreement, either all positive or negative by the 4 EIAs, were considered discrepant. WB analysis was performed on these samples to resolve the discrepancies. The discrepant samples were coded and sent blinded to Meridian Diagnostics, which performed and interpreted the WB results. All EIAs were performed and results interpreted in accordance with the manufacturers' protocols. Results for the EIAs were determined by dividing the spectrophotometric absorbance of the patient sample by the absorbance of a cutoff or calibrator control. This ratio was then used to create an index value from which a semiquantitative result was obtained. For the present study, the qualitative positive, negative, or equivocal result was used for comparison calculations of sensitivity and specificity. Sensitivity was determined as follows: number of true-positive results (as resolved by WB analysis) divided by the sum of the number of true-positive results plus the number of false-negative results, for each individual assay. The number of true-negative results divided by the sum of the number of true-negative results plus the number of false-positive results was used to calculate specificity.

WB Analysis

For WB analysis, a type-specific antibody assay described in the literature was used.[13] Briefly, antigen was extracted from HSV type 1 or HSV type 2 infected human diploid fibroblasts, electrophoresed into polyacrylamide gradient gels, and electrotransferred onto nitrocellulose membranes. Patient samples and controls were run both unadsorbed and adsorbed. For adsorption, samples first were incubated with type 1 or type 2 antigens coupled with sepharose. Three lanes then were run for each sample: an unadsorbed lane, a type 1 adsorbed lane, and a type 2 adsorbed lane. Each sample was run on a type 1 and a type 2 membrane strip for a total of 6 lanes per sample. Results were interpreted as follows: seronegative, no bands or weak bands to nonviral proteins as determined by comparison with binding patterns on strips reacted with known positive serum samples; type 1 positive, predominance of binding to the type 1 strip, or binding present on the 2 strips with an absence of reactivity with the gG-2 bands on the type 2 strip; type 2 positive, predominance of binding to the type 2 strip along with reactivity to gG-2; type 1 and type 2 positive, full antibody profile on type 1 and type 2 strips, including reactivity

with gG-2; indeterminate, atypical reactivity on either type 1 or type 2 strip or equivalent reactivity on each strip without a clear gG-2 band on the type 2 strip.

Results

The results of the samples in total agreement were combined with the results of the WB analysis–resolved samples to calculate sensitivity and specificity. Additional sensitivity studies were conducted by examining the variability of antibody titer of the 4 EIAs by diluting seropositive samples. Cross-reactivity was defined by the samples positive for a single type only by WB analysis and positive for both types by the comparative EIA.

Of the 158 serum samples tested by the 4 EIA methods, total agreement of type 1 and type 2 results were seen in only 78 (49.4%) of the samples **Table 1**. For the 80 discrepant samples, the interpretation for the WB analysis was considered the resolved or correct result and used to calculate the sensitivity and specificity of each EIA. Results from 2 samples that were indeterminate by WB analysis and the equivocal EIA results were not included in the calculations of sensitivity and specificity. There seemed to be little or no correlation of the 19 equivocal results among the 4 EIAs. Within the individual assays, there was no correlation between type 1 and type 2 equivocal results, as none of the type 1 equivocal results were equivocal for type 2, and vice versa. When comparing the equivocal results for the 4 EIAs across the board, there were only 2 cases (of 19 total) in which more than 1 assay reported an equivocal result for the same sample. In the first case, the Meridian and Wampole assays both reported equivocal results for type 1 for the same sample. This sample had a positive result by the Diamedix assay, a negative result by the Zeus assay, and was positive by WB analysis. In the second case, both Wampole and Zeus gave equivocal results for type 2 for a sample that was positive by the Diamedix assay and negative by the Meridian assay. The type 2 WB analysis result for this sample was negative. Of the 2 indeterminate results by WB analysis, the Diamedix, Wampole, and Zeus assays reported positive results for types 1 and 2. The Meridian assay reported one of these samples as positive for type 1 and negative for type 2 and the other sample as negative for type 1 and positive for type 2.

The 4 EIA methods all showed good reproducibility. By using the semiquantitative index value, the intra-assay coefficient of variation was less 7% for all 4 assays, while

Table 1
Herpes Simplex Virus IgG Antibody Type Comparison of 4 Enzyme Immunoassays With Western Blot Analysis–Resolved Results for 158 Patient Serum Samples*

		No. of Samples With Western Blot Analysis–Resolved Results		
		Positive	Negative	Indeterminate
Diamedix				
Type 1 result	Positive	86	20	2
	Negative	0	50	0
Type 2 result	Positive	60	37	2
	Negative	0	59	0
Zeus				
Type 1 result	Positive	77	19	2
	Negative	7	49	0
	Equivocal	2	2	0
Type 2 result	Positive	59	19	2
	Negative	1	73	0
	Equivocal	0	4	0
Wampole				
Type 1 result	Positive	83	22	2
	Negative	2	46	0
	Equivocal	1	2	0
Type 2 result	Positive	57	14	2
	Negative	3	79	0
	Equivocal	0	3	0
Meridian				
Type 1 result	Positive	82	3	1
	Negative	2	66	1
	Equivocal	2	1	0
Type 2 result	Positive	53	0	1
	Negative	6	95	1
	Equivocal	1	1	0

* Indeterminate and equivocal results not included in calculations of sensitivity and specificity. Diamedix, Miami, FL; Zeus Scientific, Raritan, NJ; Wampole Laboratories, Cranbury, NJ; Meridian Diagnostics (formerly Gull Laboratories), Cincinnati, OH.

interassay coefficients of variation were all less than 15%. These results were generated by running samples with high, medium, and low positive results in replicates of 5 on 3 separate days.

The Diamedix EIA showed high sensitivity, identifying 86 (100%) of 86 HSV type 1 positive samples and 60 (100%) of 60 HSV type 2 positive samples. It also was the least specific, correctly identifying only 50 (71%) of 70 type 1 negative samples and 59 (61%) of 96 type 2 negative samples. The Diamedix assay does not include an equivocal range in the result interpretation. Fifty-six of 68 samples were positive for both types by EIA but positive for only 1 type by the WB analysis–resolved results (47 type 1, 21 type 2) for a cross-reactivity rate of 82%. The cross-reactivity rate was lower among the samples that were positive for type 1 and negative for type 2 (76% [36/47]) than among samples that were negative for type 1 and positive for type 2 (95% [20/21]).

Of the samples identified as positive by WB analysis, the Zeus EIA correctly identified 77 (92%) of 84 samples as positive for type 1 and 59 (98%) of 60 samples as positive for type 2. Forty-nine of 68 samples negative for type 1 by WB analysis were identified correctly as such, yielding a specificity of 72%, and 73 of 92 samples negative for type 2 were identified correctly, for a specificity of 79%. The Zeus assay for type 1 had 4 equivocal results (2 positive and 2 negative by WB analysis). The assay for type 2 also had 4 equivocal results (all negative by WB analysis). Cross-reactivity of the Zeus EIA was seen in 37 (54%) of 68 samples positive for only 1 type by WB analysis. The cross-reactivity rate was lower for the samples that were positive only for type 1 (40% [19/47]) than for the samples that were positive only for type 2 (86% [18/21]).

The Wampole EIA correctly identified 83 (98%) of 85 samples positive for type 1 positives and 57 (95%) of 60 samples positive for type 2. For type 1 antibodies, a specificity of 68% (46/68) was calculated, while for type 2, specificity was 85% (79 of 93 samples negative for type 2 were identified correctly). The assay reported 3 equivocal results (1 positive, 2 negative by WB analysis) for type 1 and 3 equivocal results for type 2 (all negative by WB analysis). Thirty-two of 68 samples were positive for both types by EIA but positive for only 1 type by the WB analysis–resolved results, for a cross-reactivity rate of 47%. Like the Diamedix and Zeus assays, the cross-reactivity rate was lower among the samples that were positive for type 1 and negative for type 2 (28% [13/47]) than among samples that were negative for type 1 and positive for type 2 (90%) [19/21]).

For the Meridian assay, 82 of 84 samples positive for type 1 were identified correctly, resulting in a sensitivity of 98%. Fifty-three of 59 samples were identified correctly as positive for type 2, which yielded a sensitivity of 90%. The Meridian EIA had a specificity of 96% (66 of 69 correctly identified as negative) for type 1, while all 95 samples negative for type 2 (by WB analysis) were identified correctly by EIA for a specificity of 100%. Cross-reactivity was not seen in any of the 68 samples positive for only 1 antibody type by WB analysis.

Additional sensitivity studies were done with the EIAs by serially diluting 10 positive samples. The reciprocals of the highest dilutions that still gave a positive result when compared with the assay calibrator were used as the titer values **Figure 1**. Sample 29 was negative by WB analysis, as well as by the Diamedix, Wampole, and Zeus assays. Sample 7 was positive for type 1 and negative for type 2, while samples 27 and 34 were negative for type 1 and positive for type 2 by WB analysis.

Discussion

As the literature suggests, our study confirms the necessity of using specific antigenic markers, such as purified

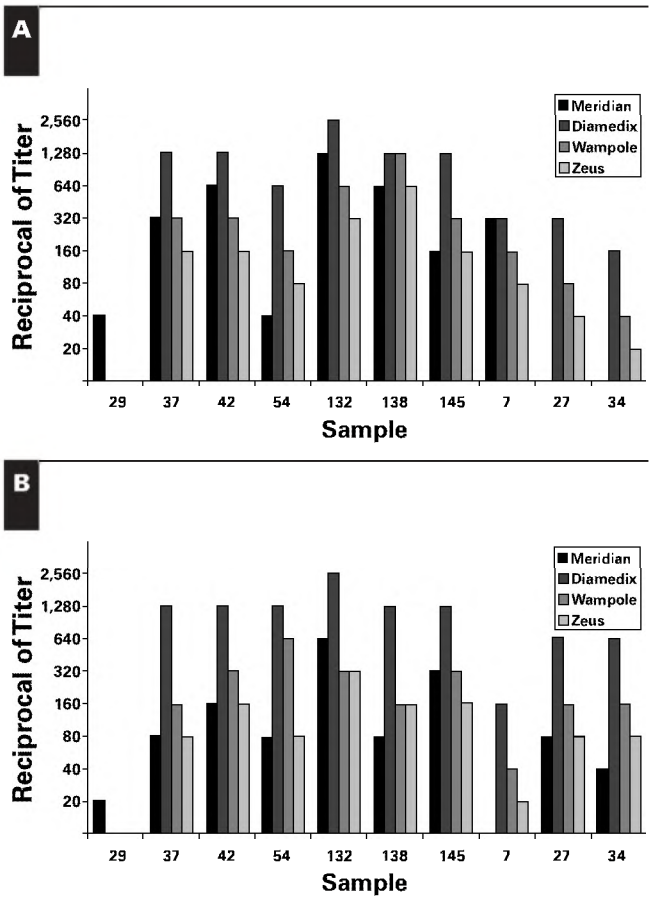

Figure 1 Comparison of titer values of 4 enzyme immunoassays. **A**, Herpes simplex virus type 1. **B**, Herpes simplex virus type 2. Meridian Diagnostics (formerly Gull Laboratories), Cincinnati, OH; Diamedix, Miami, FL; Wampole Laboratories, Cranbury, NJ; Zeus Scientific, Raritan, NJ.

glycoproteins, for accurate type-specific determination of HSV antibodies. While the 3 assays incorporating nonspecific whole viral proteins, Diamedix, Zeus, and Wampole, all showed good sensitivity for detecting HSV antibodies, ranging from 92% to 100%, they cannot differentiate adequately between types 1 and 2. This was evident in the low specificities observed for these assays, which ranged greatly, with Wampole having the highest (85%) for type 2 and Diamedix the lowest (61%) for type 2. In contrast, the purified glycoproteins used in the Meridian assay had specificities of 96% and 100% for types 1 and 2, respectively. Our calculated sensitivity of 90% for the Meridian type 2 assay was lower than the 98% in previously published data.[14]

There seemed to be little or no correlation among the 4 EIAs for the 19 equivocal results. There was no correlation between type 1 and type 2 equivocal results, as none of the type 1 equivocal results were equivocal for type 2 and vice versa. When comparing the equivocal results of the 4 EIAs across the board, there were only 2 cases (of 19 total) in which more than 1 assay gave an equivocal result for the same sample. In the first case, the Meridian and Wampole assays both reported equivocal results for type 1 on the same sample. This sample had a positive result by Diamedix and a negative result by Zeus and was positive by WB analysis. In the second case, the Wampole and Zeus assays gave equivocal results for type 2 on a sample that was positive by the Diamedix assay and negative by the Meridian assay. The type 2 WB analysis result for this sample was negative. Of the 2 indeterminate results by WB analysis, the Diamedix, Wampole, and Zeus assays reported positive results for both types 1 and 2. The Meridian assay reported one of these samples as positive for type 1 and negative for type 2 and the other as negative for type 1 and positive for type 2.

When comparing antibody titer levels of positive samples on the 4 EIAs, the Diamedix assay was consistently more sensitive (Figure 1). Of the 9 positive samples examined, the Diamedix assay always had the highest titer for both type 1 and 2 antibodies. In 16 of 18 cases, the Wampole assay was at least 1 titer higher than the Zeus assay, with the remaining 2 assays showing equivalent titers. The Meridian assay was more variable, having sometimes lower and sometimes higher titers than the Zeus and Wampole assays.

Cross-reactivity was clearly evident with the Diamedix, Zeus, and Wampole whole viral antigen–based assays, with types 1 and 2 combined rates ranging from a low of 47% for the Wampole test to a high of 82% for the Diamedix assay. These assays also were alike in that the cross-reactivity rate was higher in samples that were negative for type 1 and positive for type 2 than in samples that were positive for type 1 and negative for type 2. In contrast, the Meridian assay showed no cross-reactivity in the 65 samples that were determined by WB analysis to be positive for only 1 antibody type.

The results from our study compare favorably with evaluations of other EIAs based on the HSV type-specific purified G glycoproteins. An evaluation by Prince et al[15] of the MRL Diagnostics (Cypress, CA) immunoassay showed sensitivities for HSV type 1 and 2 antibodies of 100% and specificities of greater than 95%. These results are comparable to the results for the Meridian assay, which demonstrated slightly lower sensitivities (98% for type 1 and 90% for type 2) but slightly higher specificities (96% and 100% for types 1 and 2 antibodies, respectively).

The main difference between the 2 assays is that the MRL EIA uses recombinant gG-1 and gG-2 antigens, while the Meridian EIA uses native affinity-purified gG-1 and gG-2. Although studies have shown these type-specific tests to be superior to assays based on whole antigen preparations, there are limitations to the HSV glycoprotein G–based assays. Recombinant glycoprotein G proteins usually are derived from bacteria or baculovirus expression vectors and may not detect all the antibodies elicited in humans by exposure to native glycoprotein G. Recombinant antigens also may lack epitopes that depend on glycosylation mechanisms of mammalian cells.[8] Affinity purified glycoprotein G, therefore, is more likely to provide the complete complement of native epitopes. As with any test based on antibodies to 1 protein, not all people may produce an antibody response to the specific protein. In addition, glycoprotein G is not an essential protein for HSV viral replication, and infections may occur with glycoprotein G–deficient virus. As a result, 5% to 10% of patients may lack detectable antibody to glycoprotein G after infection.[8] Patients with early HSV infection also may have negative test results for IgG antibodies, since the IgG response to the glycoprotein G antigen arises relatively late. Studies have shown that antibody to glycoprotein G first appears 2 to 3 months after initial infection in 60% to 70% of patients, while the remaining patients may require up to 6 months to seroconvert to glycoprotein G.[8]

Even with these limitations, EIA-based tests offer many advantages. Most laboratories are well equipped with personnel and instrumentation to run these types of tests. It is a method that is recognized and used commonly and is less expensive and laborious than WB analysis. Because results are read by a spectrophotometer, the subjectivity of WB analysis band interpretation is not a factor.

The use of purified gG-1 and gG-2 from HSV types 1 and 2, respectively, as specific antigens has permitted accurate type-specific discrimination of HSV antibodies. The application of these truly type-specific markers in a commercially available microtiter plate EIA format provides a more practical and less time-consuming alternative to WB analysis, especially for testing large volumes of samples.

From the [1]Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology and the [2]Department of Pathology, Pediatrics, and Medicine, University of Utah School of Medicine, Salt Lake City, UT.

Address reprint requests to Mr Martins: ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108.

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